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In re

Patent Application of

James W. Schumm, et. al.

Serial No.: Unknown

Filed: April 20, 2001

Examiner: Unknown

I, Diane J. Frauchiger, hereby certify that this correspondence is being deposited with the US Postal Service as Express Mail Post Office to Addressee Serial No. EL453985016US addressed to Assistant Commissioner for Patents, BOX PATENT APPLICATION, Washington, D.C. 20231, on the date of my signature.

Diane J. Frauchiger  
Signature

April 21, 2001  
Date of Signature

"MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI"

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
BOX PATENT APPLICATION  
Washington, D.C. 20231

Sir:

This application is a continuation under 37 CFR 1.53(b) of U.S. Patent Application Serial No. 09/327,229, filed June 7, 1999, to be issued on April 24, 2001 as U.S. Patent No. 6,221,598, a continuation of U.S. Patent Application Serial No. 08/316,549, filed September 30, 1994, now abandoned. Prior to examination on the merits, and calculation of filing fees due with the above-identified application, please amend the subject application as follows:

**In the Specification**

Please amend the specification as follows:

On page 1, immediately before "FIELD OF THE INVENTION", please add the following new paragraph and heading:

**CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a continuation application of U.S. Patent Application Serial No. 09/327,229, filed June 7, 1999 now U.S. patent 6,221,598, which is a continuation application of U.S. Patent Application Serial No. 08/316,544, filed September 30, 1994, now abandoned.

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Replace the second full paragraph on page 3 with the following:

*Ballabio et al.* (1991), disclose a single-tube, multiplex allele-specific PCR test using two different dye-tagged fluorescent primers for detection of the  $\Delta$ F508 cystic fibrosis mutation.

On page 7, after line 18 and before line 19, add the following new paragraph:

Fig. 24 is a photograph showing the silver stained detection of the multiplex amplification in example 24.

Replace the first full paragraph on page 11 with the following:

The primers must also be designed so that the size of the resulting amplification products differ in length, thereby facilitating assignment of alleles to individual loci during detection. Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. The synthesis of the primers is conducted by procedures known to those skilled in the art.

Replace the third full paragraph on page 18 with the following:

In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMVWFA31 simultaneously in a single reaction vessel. The PCR amplifications were performed in 25 $\mu$ l volumes using 25ng template, 0.04U *Taq* DNA Polymerase/ $\mu$ l, 1x STR Buffer (50mM KCl, 10mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl<sub>2</sub> and 200 $\mu$ M each of dATP, dCTP, dGTP and dTTP), and using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 1, as described in Example 1, was employed. Eight amplification primers were used in combination, including 1 $\mu$ M each HUMCSF1PO primer 2 [SEQ. ID. 6] and fluorescein-labeled